HYPERVITAMINOSIS D IN MONKEYS;

A CLINICAL AND PATHOLOGIC STUDY *

SIDNEY P. KENT, M.D.; GORDON F. VAWTER, M.D.; ROBERT M. DOWBEN, M.D., and RICHARD E. BENSON, Capt., USAF (VC)

From the Department of Pathology, Medical College of Alabama, Birmingham, Ala.; the Department of Pathology, Children's Hospital, Boston, Mass.; the Department of Medicine, Northwestern University, Chicago, Ill.; and the School of Aviation Medicine, USAF, Randolph Air Force Base, Texas

The Radiobiological Laboratory of the University of Texas and the United States Air Force, Austin, Texas, and the School of Aviation Medicine, Randolph Air Force Base, Texas, maintain a monkey colony in connection with an experimental program in radiobiology. Because of an error by the manufacturer of the monkey food, the entire colony received excessive amounts of calcium, phosphorus, and vitamin D for a period of nearly three months. Acute vitamin D intoxication developed and resulted in a number of deaths before the diagnosis was established. This opportunity of observing vitamin D intoxication in a large number of higher mammals was not a planned experiment, but developed accidentally. The clinical and pathologic observations made while the colony received the toxic diet and for one year thereafter are the subject of this report.

CLINICAL INFORMATION

At the onset of the high vitamin D diet, there were 558 monkeys (*Macaca mulatta*) in the colony: 283 were in the Austin colony and 275 were in a holding colony at Randolph Air Force Base. Of the 283 animals in the Austin colony, 103 had received whole-body ionizing radiation; 70 had received focal ionizing radiation to the right eye; 21 had received intravenous nitrogen mustard; 89 were control animals which had received neither ionizing radiation nor nitrogen mustard. Thirty-two of the irradiated animals had had positive skin tests for tuberculosis and had received 250 mg. of streptomycin and 59 mg. of isoniazid intramuscularly twice a week (November 2, 1953 to January 15, 1956). All of these animals were being studied to observe the latent effects of ionizing radiation or nitrogen mustard. None of the 275 animals in the Randolph colony had been utilized in the experi-

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mental program of the Laboratory. Because the data on the Austin colony are more complete, only they are reported unless otherwise specified.

The animals varied in age from 3 to 9 years, and in weight from 2.5 to 10.0 kg. Seven of the 283 animals were female; only males are used in the experimental program of the laboratory. The animals were maintained on a daily diet consisting of approximately 114 gm. of monkey meal (whole wheat flour, 47 per cent; soy bean oil, 17.5 per cent; powdered milk, 5 per cent; bone meal, 5 per cent; molasses, 4 per cent; wheat germ meal, 4.5 per cent; cornmeal, 10 per cent; salad oil, 5 per cent; salt, 1 per cent; irradiated yeast, 1 per cent), supplemented with fresh fruit (oranges, grapefruit, apples, or bananas) and vegetables (carrots, lettuce, potatoes, or cabbage) three days per week. Because of the aforementioned error in food manufacture, the diet of the colony was altered so as to include 162,000 U.S.P. units of vitamin D per animal per day (Table I). Each also received approximately

TABLE I
*Analysis of Monkey Meal**

		A†	B†	C†
Total solids	Per cent	91.43	90.88	89.34
Moisture	Per cent	8.57	9.12	10.66
Protein	Per cent	22.19	19.42	20.17
Fat	Per cent	7.38	7.97	4.59
Ash	Per cent	6.92	7.83	5.16
Fiber	Per cent	2.81	2.85	2.40
Carbohydrate	Per cent	52.13	52.81	56.98
Calcium	Grams (per animal, per day)‡	1.54	3.50	0.87
Phosphorus	Grams (per animal, per day)	0.72	2.90	0.62
Vitamin D	U.S.P. units (per animal, per day)	4,252	163,000	40.00

* Analyses were performed by the Food Research Laboratory, Long Island City, N.Y., and by the Fourth U.S. Army Medical Laboratory, Fort Sam Houston, Texas.

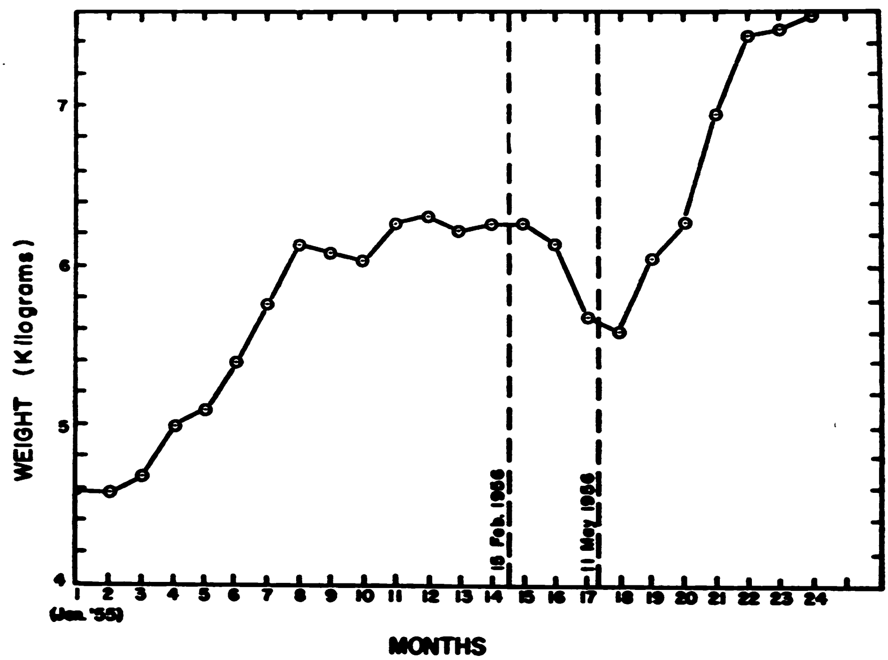
† Diet A—Standard monkey diet fed prior to high vitamin D diet.
Diet B—High vitamin D diet.
Diet C—Diet fed subsequently; beginning on August 11, 1956, each animal received a vitamin supplement containing approximately 400 units of vitamin D daily.

‡ Each animal received approximately 114 grams of the monkey meal per day.

3.5 gm. of calcium and 2.9 gm. of phosphorus per day. The animals in the Austin colony received the high vitamin D diet for 81 days (February 15 to May 7, 1956), and the animals in the Randolph colony for 86 days (January 23 to April 20, 1956) before the error was discovered and the diet was changed. On April 20, 1956, the animals in the Randolph colony, and on May 7, 1956, the animals in the Austin colony, were placed on a diet low in vitamin D. The amount of calcium and phosphorus in the diet was also reduced. In addition, each animal in the Austin colony received 50 mg. of Diamox (acetazoleamide) and 4 cc. of aluminum hydroxide gel per day for 60 days in an attempt to maintain a slight acidosis. It was thought that this might tend to lessen the deposition of calcium in the tissues and promote its excretion. Antibiotic agents (penicillin, streptomycin, or chloromycetin) were used in the treatment of respiratory and gastrointestinal infections. A vitamin supplement was added to the diet on August 11, 1956, so that thereafter each animal would receive a total of 400 U.S.P. units of vitamin D daily.

The first clinical evidence of hypervitaminosis D was an increased incidence of upper respiratory infection and diarrhea occurring during late April and early May 1956. This was greater than was usually noted during this period of the year.

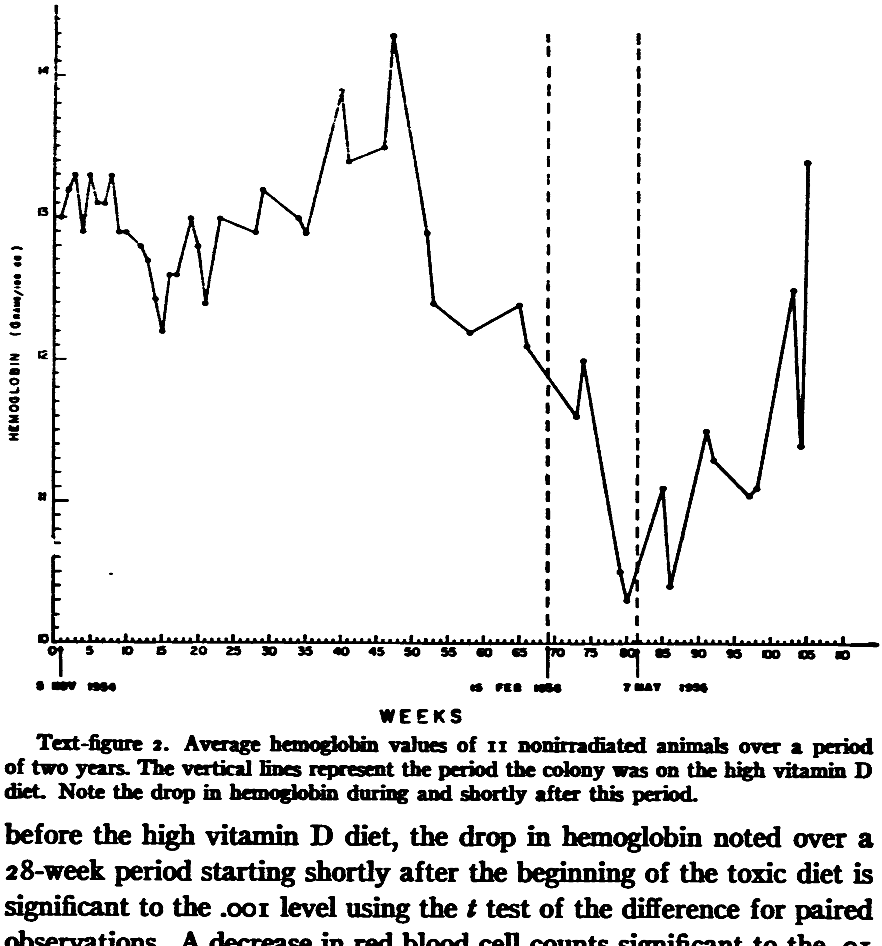
Each animal in the colony is weighed monthly. A graph of the average weight of 20 nonirradiated control animals over a period of 2 years, indicates the onset of weight loss shortly after the inception of the high vitamin D diet (diet B, Text-fig. 1). The weight loss con-



Text-figure 1. Weight of 20 nonirradiated control animals over a period of two years. The vertical lines represent the period the colony was on the high vitamin D diet. Note the weight loss during and shortly after that period.

tinued for approximately one month after diet B was terminated. Immediately following this, the entire group of animals gained weight progressively. A similar weight loss was also noted in the colony as a whole during the high vitamin D diet.

A peripheral blood count and smear are obtained on selected animals in the colony every 2 weeks to 1 month. Most of these animals had been irradiated. However, a group of nonirradiated control animals was available for study. An appreciable decrease in erythrocytes and hemoglobin during and after the period of excess vitamin D intake was evident (Text-fig. 2). When compared to the period immediately



Text-figure 2. Average hemoglobin values of 11 nonirradiated animals over a period of two years. The vertical lines represent the period the colony was on the high vitamin D diet. Note the drop in hemoglobin during and shortly after this period.

before the high vitamin D diet, the drop in hemoglobin noted over a 28-week period starting shortly after the beginning of the toxic diet is significant to the .001 level using the *t* test of the difference for paired observations. A decrease in red blood cell counts significant to the .01 level was noted during the same period. Similar hematologic findings have been described in human patients who have received high doses of vitamin D over long periods of time.[2]

Blood urea nitrogen (BUN), calcium, and phosphorus determinations on the serum of 40 monkeys were done within two weeks after the high vitamin D diet was terminated and repeated approximately 9 months after termination of the episode. The average BUN for the groups on the first examination was 26.3 (15 to 37) mg. per hundred milliliters and 9 months later was 19.4 (11 to 24) mg. When compared to the average BUN of 10 "normal" animals, 19.8 (16 to 24) mg.,* this information suggests that a minor degree of renal failure was present in many of the animals near the end of the high vitamin D diet and that the renal function improved during the succeeding period of several months. The average serum calcium level initially (May 18, 1956) was 13.4 (11 to 17) mg. per hundred milliliters and the average serum phosphorus was 4.7 (3 to 7) mg. Nine months later, the average serum calcium value was 12.2 (10 to 14) mg., and the serum phosphorus, 6.8 (4 to 8) mg. This may be compared to values for calcium of 11.36 mg. and for phosphorus of 3.7 mg. given by Wats and Das Gupta[3] for "normal" *Macaca mulatta*. Similar elevations of BUN, calcium, and phosphorus have been described both in human subjects and in experimental animals receiving high vitamin D diets.[4-6]

Electrocardiographic tracings were done on 12 animals shortly after diet B was terminated. Two significant alterations were noted. In some, the T waves were rounded, a feature consistent with the effect of hypercalcemia. Large peaked T waves as seen with hyperpotassemia were also noted. Because of the very rapid heart rate natural in monkeys and their excitement from being handled, evaluation of an abnormality of the QT interval was extremely difficult.

Roentgenograms were made of the torso, including the proximal ends of the humeri and femurs in selected animals. These were obtained at the time the toxic diet was stopped, and at monthly intervals for 6 months. In the long bones no changes characteristic of prolonged vitamin D intoxication were seen. Soft tissue calcifications were not noted in the films, although, as will be shown later, they were observed in necropsy specimens obtained from animals dying during this period.

Catheterized urine specimens were procured from several animals immediately after the high vitamin D diet was terminated. These showed a large amount of sediment consisting chiefly of calcium phosphate crystals. Leukocytes, red blood cells, epithelial cells, and granular casts were also found.

Approximately a month after the high vitamin D diet was terminated, the surviving animals in the colony appeared to be in good health, and no symptoms which could be related to the high vitamin D diet were noted in the period that followed.

Pathologic Study

The following data are based on a study of 114 animals from the Austin colony that died or were sacrificed between June 20, 1955 and May 7, 1957. Twenty-six of these monkeys died between June 20, 1955

* Obtained from the Los Alamos Scientific Laboratories, Los Alamos, New Mexico.

and February 14, 1956, while the colony received the nontoxic diet A (Table I). These animals served as controls. Thirty-one died between February 15, 1956 and May 7, 1956, while the colony subsisted on diet B (high vitamin D). Fifty-six animals died while receiving diet C during the year following the discontinuance of the high vitamin D diet. Most of the tissue procured at necropsy from the 110 animals that died in the Randolph colony from the day that group of animals received diet B until one year later (January 23, 1956 to January 23, 1957) was also studied. The pathologic changes in the tissues from the two groups were very similar. Since the material from the Austin colony was more suitable, it only is used for this report unless otherwise noted.

All of the animals reported were necropsied and tissues were taken for microscopic examination from the parotid gland, submaxillary gland, esophagus, stomach, small and large intestine, pancreas, liver, larynx, trachea, lung, heart, aorta, kidney, urinary bladder, prostate, testis, adrenal, thyroid, pituitary, cerebrum, cerebellum, sternum, rib, vertebrae, head and shaft of the femur, lymph nodes, spleen, skin, and skeletal muscle. The eyes were examined in more than half and the parathyroid glands were available in more than three fourths of the animals. Tissues were fixed in buffered neutral formalin, embedded in paraffin, and sectioned at 6 microns thickness. Sections were stained with hematoxylin and eosin. The von Kossa method was used in demonstrating calcium in tissue.[7] Von Kossa stains were prepared on sections of the kidney, heart, and lung of all the animals reported. In 14 animals dying at the height of the vitamin D feeding episode, von Kossa stains were done on all the tissue. The alizarin red method for staining calcium[7] was applied to occasional tissues. Because the distribution of calcium was shown to be similar by the two methods, the alizarin red method was not used on all tissues. Gomori's method[8] for demonstrating iron in tissue was used regularly.

Results

The characteristic lesions found in the animals with hypervitaminosis D consisted of mineral deposits with or without associated inflammation, depending on the tissue involved. The term "mineral deposits" is used since calcium and iron were found in all of these lesions, and phosphorus was probably present in most (Table II). Other minerals may have been present, but no attempt was made to demonstrate them. In addition, an organic component was present in the lesions, particularly in the kidneys. This consisted of an amorphous, eosinophilic material which was positive with the periodic acid-Schiff (PAS) stain, metachromatic with toluidine blue and stained blue with Alcian

TABLE II

*Chemical Analysis of Kidney, Heart, and Lung for Calcium and Phosphorus**

Organ	Control animals			Hypervitaminosis D animals		
	Animal number	Calcium†	Phosphorus†	Animal number	Calcium†	Phosphorus†
Kidney	112+	2.63	2.90	V-5	10.16	3.03
	K-8	1.46	2.90	370+	8.83	4.53
	309+	3.10	2.30	Z-5	19.67	7.00
	127	0.96	6.00	116	6.00	10.80
	489	2.40	7.08	133	12.00	9.60
	686	0.48	8.76	61+	6.72	7.80
Average		1.84	4.99		10.56	7.13
Heart	112+	0.24	4.92	V-5	1.97	2.63
	K-8	1.80	0.97	370+	3.30	4.67
	309+	5.28	4.80	Z-5	1.20	3.00
	127	3.60	4.44	116	5.28	8.64
	489	3.84	4.20	133	4.44	4.56
	686	2.88	5.76	61+	2.16	4.92
Average		2.94	4.18		3.06	4.74
Lungs	112+	2.97	2.23	V-5	4.57	2.43
	K-8	1.43	2.20	370+	3.60	2.30
	309+	3.60	2.60	Z-5	2.40	6.96
	127	5.78	7.92	116	5.28	8.64
	489	2.46	6.48	133	3.84	6.00
	686	3.60	6.00	61+	6.96	2.40
Average		3.31	4.57		4.44	4.79

* All determinations were done at the Fourth U.S. Army Medical Laboratory, Fort Sam Houston, Texas, under the supervision of Capt. Arthur C. Dixon.

† All values given as mg. of calcium or phosphorus per 100 mg. of dried tissue.

blue. These characteristics suggest that this material contained an acid mucopolysaccharide. A similar material has recently been described in the soft tissue lesions of rats receiving high doses of vitamin D.[9]

The livers of two animals dying within one month after diet B was terminated, were assayed for vitamin D and compared to similar assays on two control animals and two animals which died 9 months after the termination of diet B (Table III). The vitamin D content in the first two animals was high as compared to the controls. The concentration of vitamin D in the last two animals was near the control level.

Urinary Tract

Characteristic lesions were found more consistently in kidneys than in any other tissue. An animal which died 28 days after the beginning of diet B was the first to show focal mineral deposits. The kidneys of every animal that died thereafter manifested some degree of mineralization.

TABLE III
*Analysis of Liver for Vitamin D**

Animal No.	Group 1†		Group 2‡		Group 3§	
	a	b	1	2	167	639
U.S.P. units of vitamin D per gm. of fresh liver	3.5	1.4	11	10	2.1	4.2
Average	2.45		10.5		3.15	

* Bio-assays performed by Food Research Laboratories, Inc., Long Island City, N.Y.
† Two "normal" animals obtained from Okatie Farms, Hardieville, S.C.
‡ Two animals dying within one month after diet B was terminated.
§ Two animals dying nine months after the termination of diet B.

The earliest lesions (28 to 54 days) consisted of occasional small deposits in the tubules, often in the basement membranes. Many were not associated with an inflammatory reaction. The number of deposits increased with time so that by the 76th day, severe lesions were noted (Fig. 3). In these cases the deposits could be seen on gross examination as yellowish-white flecks in the cortex and medulla (Fig. 1). They could also be seen in postmortem roentgenograms of the kidneys (Fig. 2).

Microscopically, the deposits appeared in the tubules for the most part or in the surrounding interstitial tissue. The distal convoluted, proximal collecting, and thin loop tubules were involved most frequently. The proximal convoluted tubules and medullary collecting tubules were relatively spared. In the sections stained with hematoxylin and eosin, the deposits varied from a dark or light blue to an almost colorless appearance. Calcium and iron were demonstrated consistently in them. Foreign body type giant cells and mononuclear cells were often found in and about the deposits, and polymorphonuclear cells were noted occasionally (Fig. 4). Many of the lesions were metachromatic when stained with toluidine blue. They were also PAS positive and stained blue with Alcian blue before and after decalcification. In several cases, deposits were noted in the glomeruli and also in the walls of small arteries and veins. Hyaline casts were often present in the tubules. In some instances, the tubules above the

TABLE IV

Distribution of Calcium Deposits in Heart, Kidneys, and Lungs

Organ	Animal number									
Heart	213+	551	54+	C-8	55+	X-7	233	61+	86+	165+
Left ventricle										
Apex	++*	o	+	++++	++	+	+	†	+	o
Base	+++	+	o	+++		+	o	+	+	
Middle	++++	++	+	++++	++	+	+	+	+	o
Right ventricle										
Apex	o	o	o	+++	o	o	o	o	o	o
Base	+	o	o	++	o	o	o	o	o	o
Left atrium	+	o	o	++	++	o	o	o	o	o
Right atrium	o	o	o	o	o	o	o	o	o	o
Kidney										
Superior pole										
Right	+++	+++	++	++	+++	+++	+++	+++	+++	+
Left	+++	++++	++	++	+++	+	+++	+++	+++	+
Inferior pole										
Right	+++	+++	+++	++	+++	+++	+++	++	+++	+
Left	+++	++++	+++	++	+++	+	+++	+++	+++	+
Middle										
Right	+++	++++	+++	++	+++	+++	+++	+++	+++	+
Left	+++	+++	+++	++	+++	+	+++	+++	+++	+
Lung										
Upper lobe										
Right	++++	+++	+++	++	++	++	+	++	+	o
Left	++++	+++	+++	++	++	++	+	++	+	o
Middle lobe										
Right	+++	+	++	++	++	++	+	++	+	o
Left	++++	+	++	++	++	+	+	++	+	o
Lower lobe										
Right	++++	++	++	++	++	++	+	++	+	o
Left	++++	++	+++	++	++	++	+	++	+	o

* The quantity of calcium was estimated on the basis of the amount of black precipitate in the von Kossa stained sections: ++++ = most severe calcification noted in a given organ, +++, ++, + = 75, 50, 25 per cent of the maximum.

† Tissue not available for examination.

mineral deposits were dilated. Multiple sections were taken from both kidneys of 10 animals dying at the height of the vitamin D feeding episode. Although there was some variation in the number of calcium deposits from slide to slide, no one portion of the kidney was more consistently involved than another (Table IV).

The degree of calcification in the kidneys seemed to decrease about a month after diet B was terminated and continued to do so throughout the remainder of the year. In addition to a decrease in the number of deposits, there was a decrease in the amount of inflammatory reaction as well. For the last half of the year, no inflammation was noted in relation to the renal deposits. One animal dying in the Randolph colony approximately 6 months after the end of the high vitamin D diet showed renal lesions very closely resembling those found in human patients with malignant hypertension. As this was the only animal in which such lesions appeared, it is difficult to relate them to the high vitamin D diet.

The renal pelvis and bladder and the testes were not abnormal nor were stones found in the urinary tract. In one animal, fine granular deposits of calcium were noted in the stroma of the prostate in relation to the elastic fibers, and in two cases small deposits were found in the epithelium lining the seminal vesicles.

Respiratory System

The lungs were often affected but not as regularly as the kidneys: mineral deposits were found in the lungs of 23 of the 39 animals dying between the 55th to 208th day. The early lesions appeared in the mucosa of the small bronchi and alveolar ducts where mineral deposits frequently lay within the basement membranes. The lesions contained both calcium and iron and were often surrounded by mononuclear and foreign body giant cells. The granulomas caused deformity of the overlying epithelium in some cases and occasionally resulted in ulceration. In the animals dying near the 81st day, the deposits were more extensive. In these, the alveolar walls were incrusted with calcium and iron (Fig. 5). Also multiple small granulomas containing calcium and iron were found in the mucous membranes of the bronchi, trachea, and larynx, on and around the basement membranes. Mineral deposits were not found in animals dying in the Austin colony after the 281st day. However, 3 animals from the Randolph group that died almost one year after diet B was terminated showed extensive calcifications. Some of the deposits occurred in the lumens of the alveoli.

In the older control animals, the laryngeal cartilages were often calcified, but this was not the case in the bronchi. In the animals that received the high vitamin D diet, the bronchial cartilages were frequently calcified. The first animal to show this, died on the 68th day. Twenty of the 29 animals dying between the 68th and 230th days had some calcification of the bronchial cartilages. Presumably this was related to the high vitamin D diet.

Cardiovascular System

Cardiac lesions were noted only in the animals dying between the 55th and 86th days: 9 of the 24 animals dying during this period showed lesions. Abnormality was recognized grossly in all 9 animals. This consisted of a scattered grayish-yellow mottling of the myocardium (Fig. 6). Microscopically, the lesions were focal and exhibited calcium and iron deposits. The deposits consisted of fine granules or irregular plaques along the muscle fibers. Evidence of myofibril degeneration and necrosis was found in these areas (Fig. 8). Mononuclear cells also were often found, although the inflammatory reaction was not marked. Foreign body giant cells were seen only occasionally. In the animals with minimal myocardial damage, the lesions were predominantly subendocardial in location. In the more severe cases, lesions were found throughout the myocardium of the left ventricle (Fig. 7). In these cases, intramyocardial vessels were often mineralized, sometimes partially necrotic and occasionally the seat of thrombosis. Multiple sections of the heart in the 9 cases showed that the lesions were always in the left ventricle, occasionally in the left atrium and right ventricle, but never in the right atrium (Table IV).

Focal areas of scarring and chronic myocarditis were found in the left ventricle of 2 animals dying almost a year after the termination of the high vitamin D diet. Although no calcium was seen in these lesions, their distribution suggested that they might represent the residuals of myocardial damage similar to that seen in animals dying between the 55th and 86th days.

Mineral deposits were also found in the aortas of 12 of 34 animals dying between the 55th and 140th days. The deposits were predominantly in the media along the elastic fibers. The lesions were not seen on gross examination and there was no loss of aortic elasticity. In animals from Randolph Field, gross aortic calcifications were found twice, and in the aorta from a third case, a calcified area was surrounded by a dense infiltration of neutrophils. This represented the sole instance of aortic mineralization associated with inflammation.

If mineralization of small vessels in the presence of massive parenchymal calcification be excluded, disseminated lesions of small arteries in soft tissues were encountered in only 3 animals: one animal from the Randolph Field colony and one from the Austin colony died near the end of the high vitamin D diet. The third animal was from the Randolph group and died 11 months after the termination of diet B. The deposits lay along the internal elastic laminas in all cases (Fig. 9).

Digestive Tract

Next to renal involvement, the earliest and most common evidence of calcification was found in the salivary glands: 27 of 43 animals dying between the 47th and 226th days had these lesions. The submaxillary glands were involved almost twice as commonly as the parotid glands. The deposits appeared in the walls or lumens of small ducts (Fig. 10). Duct dilatation was found occasionally, in both the presence and the absence of demonstrable ductal obstruction.

Mineral deposits were noted in the mucosa of the stomach of 10 animals. In most cases they were scanty, but in one they were extensive (Fig. 12). In the latter, the muscularis mucosae was involved but not the other muscle layers. The mineral deposits were often associated with foreign body granulomas. Similar mineral deposits were noted in the jejunum of one animal and the colons of 2 animals. In all 3 cases, the deposits were slight and not associated with an inflammatory reaction.

Lesions attributable to hypervitaminosis D were not found in any of the sections taken from the tongue, esophagus, liver, and pancreas.

Central Nervous System

Several months after the termination of diet B, 6 animals which had received high doses of x-ray irradiation to the head died. Necrotic areas were found in the path of irradiation through the brain. Calcium and iron deposits were found in these. Similar deposits have been noted in monkeys receiving focal ionizing irradiation to the head in the absence of a high vitamin D diet.[10] Other evidences of calcification were not noted in the central nervous system.

Endocrine Glands

Although sections were examined from the pituitary, thyroid, and pancreas in all cases, no mineral deposits were noted in these areas. Laminated deposits were frequently found in the inner portion of the adrenal cortex (Fig. 11), but these were present equally frequently in the control animals.

Although the parathyroid glands were not weighed, they did not appear to be enlarged. They were composed characteristically of uniform cells with dark-staining nuclei and scant cytoplasm. The average diameters of 50 cells from 4 control animals and 4 animals dying at the height of the vitamin D episode were compared. There was no significant difference between the 2 groups as has been described in the dog.[11]

Musculo-skeletal System

There was little evidence of destruction in the sections of bone examined. In one animal the lacunas in the vertebrae were larger than those seen in other cases; however, there was no evidence of an increase in the number of osteoblasts, nor was there any gross evidence of demineralization. The paucity of bone lesions in these animals may be due to the high calcium and phosphorus content of the diet. The bone marrow in a number of cases was atrophic, although this could be related either to high doses of whole-body ionizing radiation or to the administration of nitrogen mustard. Mineral deposits were not noted in the sections of skeletal muscle which were usually taken from the sartorius muscle.

Other Tissues

Although sections were examined from the skin, testes, spleen, lymph nodes, and occasionally from the eyes, mineral deposits as perceived in the other soft tissues were not found.

Discussion

Since hypervitaminosis D was first noted in 1928, it has been described in a number of species, including man.[12-14] Only two reports were found in the literature dealing with this condition in monkeys. Hess and Lewis[15] produced it in monkeys but they were interested primarily in the blood calcium and did not describe the pathologic lesions. The 19 monkeys reported by Cowdry and Scott[16] apparently received more vitamin D than our animals. Despite this, the mineral deposits in the soft tissue were minimal. The high calcium and phosphorus content of the diet of our animals may explain the extensive deposits found in the soft tissue of many.

The quantity of vitamin D received by the animals included in this report was well above the toxic dose. Despite this, 28 days elapsed before any pathologic lesions attributable to hypervitaminosis D appeared in the tissues. It was 55 days before really severe lesions were noted. The alterations were most severe in animals dying about the time the high vitamin D diet was terminated and for one month thereafter. The amount of calcification and the number of organs affected decreased with time, so that animals dying one year after the termination of diet B showed slight mineralization. The animals dying in the Randolph Field colony generally revealed more severe lesions. This was particularly evident in the necropsy examination of animals during the last half of the year following the termination of diet B. Three animals from the Randolph group during that period had extensive mineral deposits in the lungs, as well as in the kidneys. Two factors probably contributed to the difference in the two groups: first, the Randolph group was on diet B for 5 days longer; second, the Randolph group was housed outdoors and was thus exposed to the sun, whereas most of the Austin colony was maintained indoors.

The distribution of the lesions in these monkeys was similar to that described in other species. However, the degree of vascular involvement seemed to be less than that described in rodents.

The reaction of the tissues to the mineral deposits varied considerably. The deposits in the kidneys, mucosa of the stomach, and the mucosa of the respiratory tree usually were surrounded by mononuclear cells and foreign body giant cells forming small granulomas which distorted the tissues. On the other hand, there was usually no inflammatory reaction to the mineral deposits in the aorta and salivary gland. The muscle fibers in the heart containing mineral deposits showed evidence of degeneration or were frankly necrotic. A mononuclear cell reaction was frequently seen in the myocardium but was usually mild, and foreign body giant cells were observed only occasionally.

The reports in the literature dealing with the pathologic alterations of hypervitaminosis do not mention the presence of inorganic iron in the lesions as observed in our animals. The presence of iron in the mineral deposits raises the possibility that there was systemic disturbance of iron metabolism in addition to that of calcium and phosphorus. This is not likely, for as Bunting[17] has pointed out, iron is usually found in soft tissue calcifications regardless of the cause of the lesions.

Mulligan[18] has suggested that the tissues where acids are formed, are rendered alkaline when these acids are secreted so that calcium deposition is favored. The high incidence of calcification of the acid-secreting kidneys, mucosa of the stomach and lungs, and the absence of calcification of the alkaline-secreting pancreas and liver in our animals adds support to this idea. The tendency for the calcifications to be greater in the left ventricle and atrium than in the right ventricle and atrium, as noted in our animals and as has been reported in other species, may be explained on the same basis; that is, the pH of the arterial blood is higher than the venous blood.

Most of the animals reported on here which died spontaneously, succumbed as a result of acute infection of the lungs or colon. Though the toxic amounts of vitamin D may have lowered the resistance of the colony to infection, it is not unusual to have monkeys in the colony die of pneumonia or acute colitis, particularly in the spring. Some of the animals apparently died of hypervitaminosis D. In these, the calcification and degeneration in the myocardium were extensive, and there was evidence of congestive heart failure: dilatation of the ventricles, congestion and edema of the lungs, and congestion of the liver. The renal lesions in a number of the animals were extensive, and renal failure was probably a lethal factor. It is difficult to evaluate this, as studies of renal function were not done on any of the animals that died. Blood urea nitrogen determinations were done on 40 animals which did not die, and the levels were elevated in many.

Because many of the animals had received some type of ionizing radiation or nitrogen mustard, the relationship to hypervitaminosis of some of the pathologic lesions found at necropsy was doubtful. Hypoplasia of the bone marrow, atrophy of the spleen, lymph nodes, and thymus are features attributable to radiation or nitrogen mustard administration and have not been described in hypervitaminosis D in other species. When these lesions were found in our animals, they therefore could be related in every case to either ionizing radiation or the administration of nitrogen mustard. On the other hand, widespread soft tissue mineralization accompanied by inflammation or degeneration is not an anticipated reaction to irradiation or nitrogen mustard. When appearing in animals receiving a diet high in vitamin D and not in the control animals, this obviously reflects the effect of the high vitamin D diet.

Summary

A colony of 558 monkeys (*Macaca mulatta*) was inadvertently given a diet high in vitamin D for a period of approximately 3 months. The clinical and pathologic findings noted in these animals during the period of the high vitamin D diet and for a year thereafter have been described, and compared to those reported in other species. There were weight loss, anemia, elevation of blood urea nitrogen and serum calcium, and an increased incidence of diarrhea and upper respiratory infections during the period of acute exposure. Lesions were most common in the kidney, salivary gland and lung, and consisted of calcium and iron deposits which were often associated with a foreign body type reaction. The animals examined a year after the high vitamin D diet was terminated showed few lesions.

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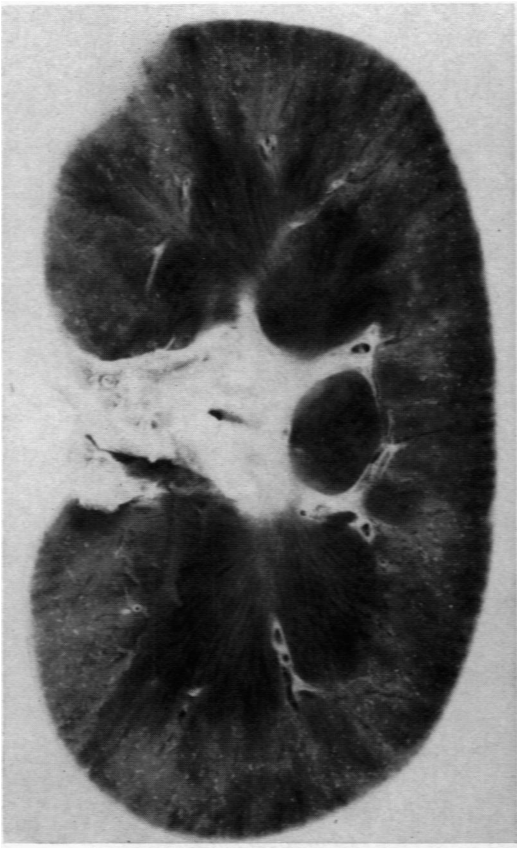
LEGENDS FOR FIGURES

FIG. 1. Kidney. White flecks of calcium are evident, particularly in the cortex. × 2.5.

FIG. 2. Postmortem roentgenogram of the kidney. Note the small opacities throughout the kidney. × 2.5.

FIG. 3. Kidney. Mineral deposits are widely distributed. Von Kossa stain. × 6.



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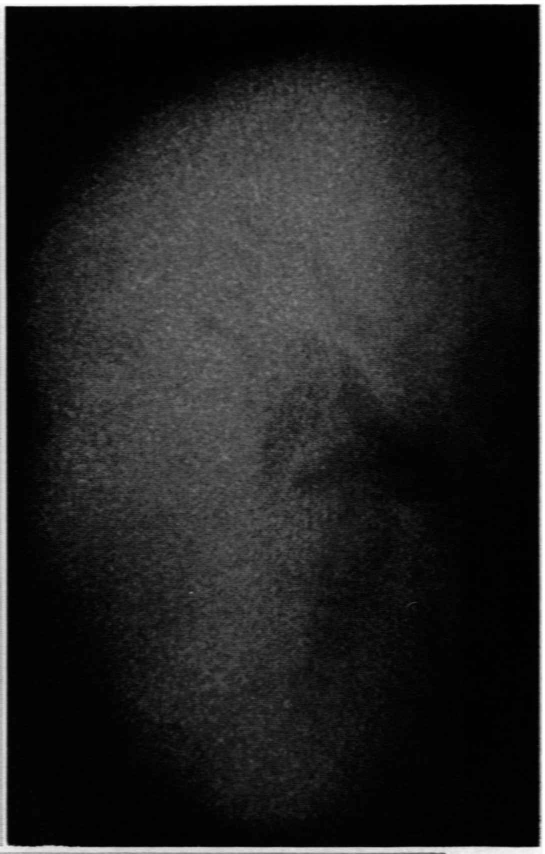

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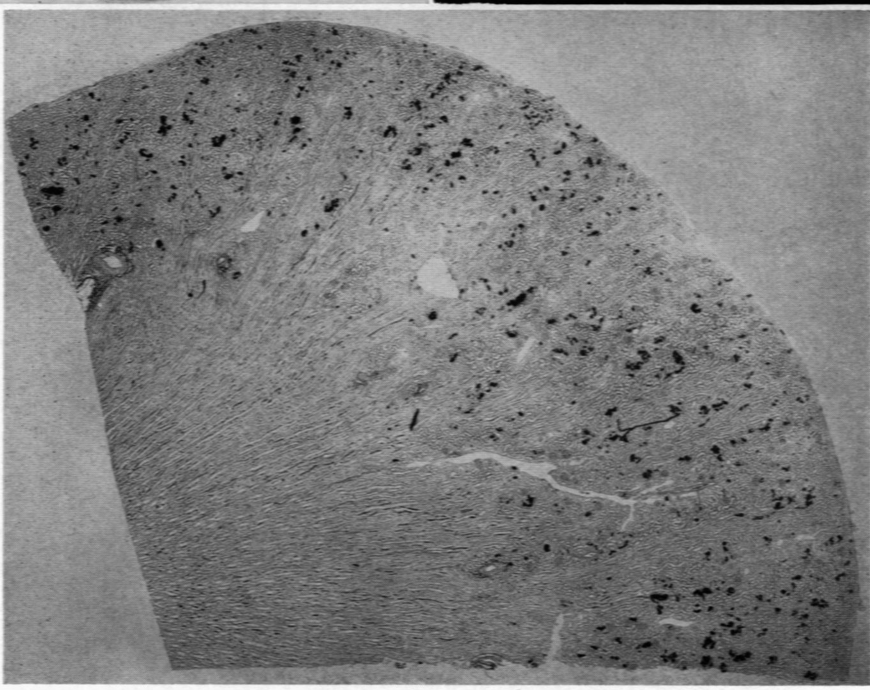

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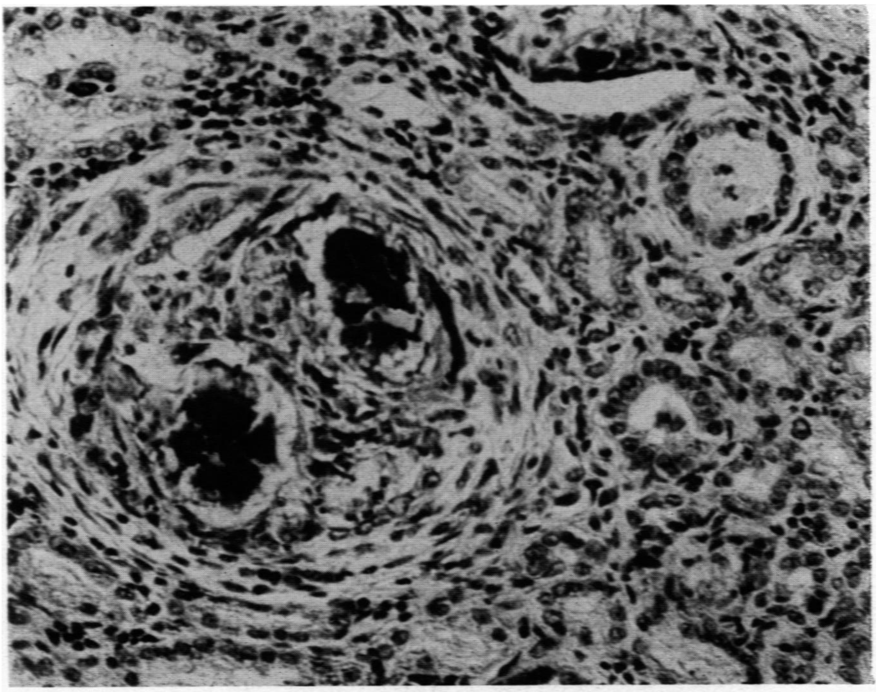
FIG. 4. Kidney showing granulomatous reaction to the mineral deposits. Hematoxylin and eosin stain. × 250.

FIG. 5. Lung showing extensive calcium deposits. Von Kossa stain. × 6.



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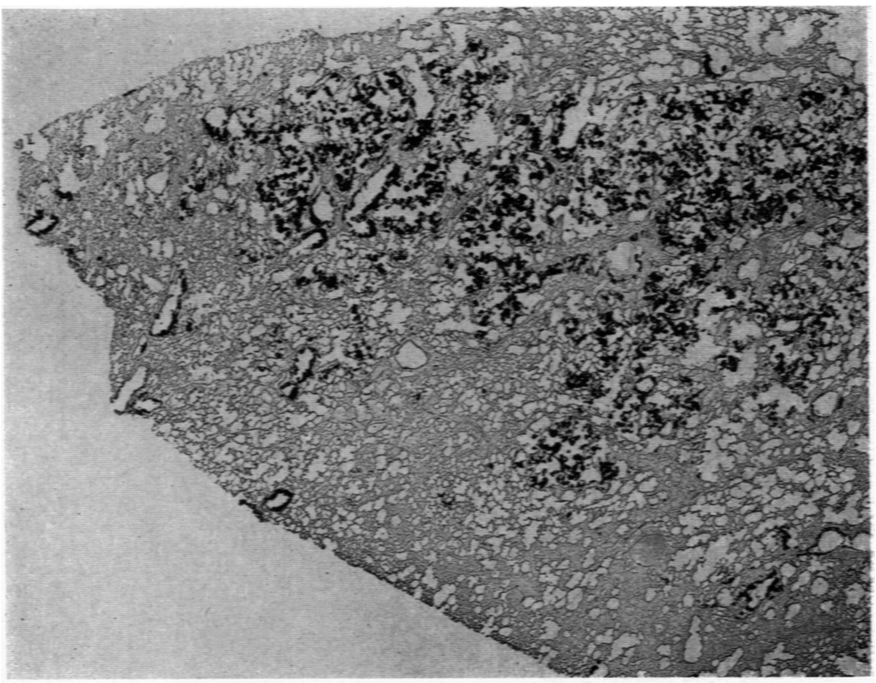

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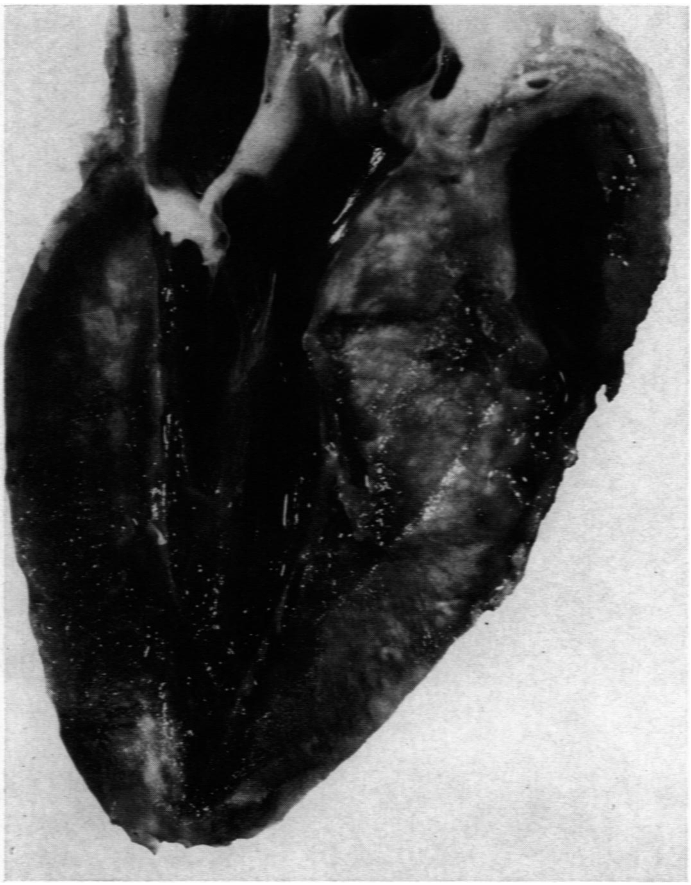
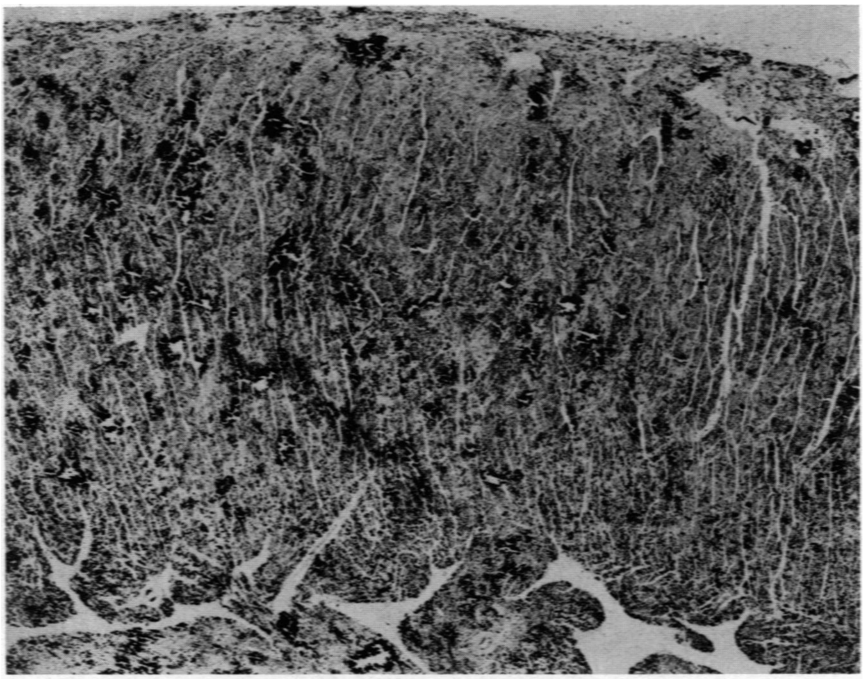


FIG. 6. Heart. Note the mottled appearance of the myocardium. × 2.5.

FIG. 7. Heart. Extensive calcium deposits are found throughout the myocardium. Von Kossa stain. × 12.

FIG. 8. Heart showing calcification and atrophy of muscle fibers. Hematoxylin and eosin stain. × 93.



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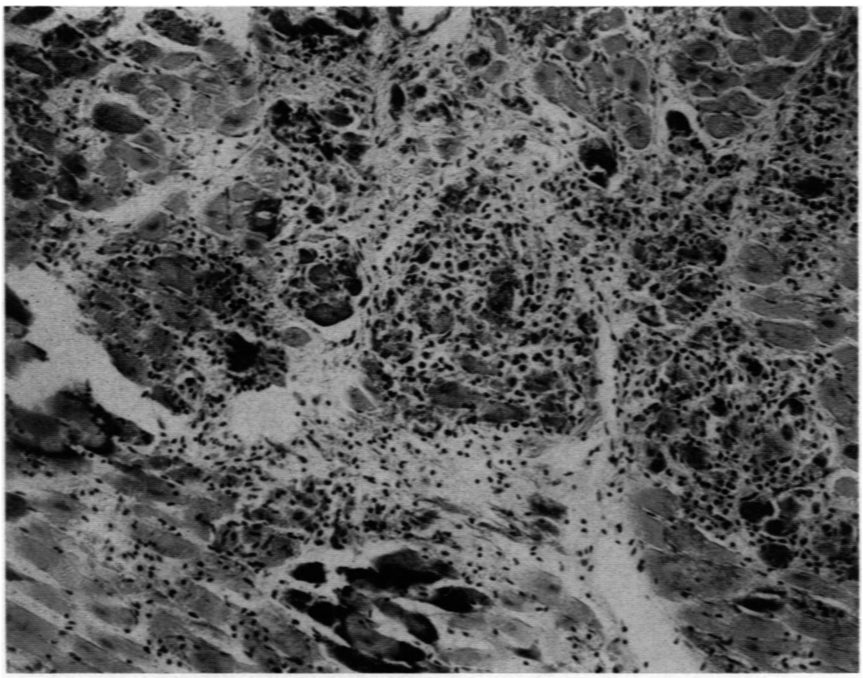

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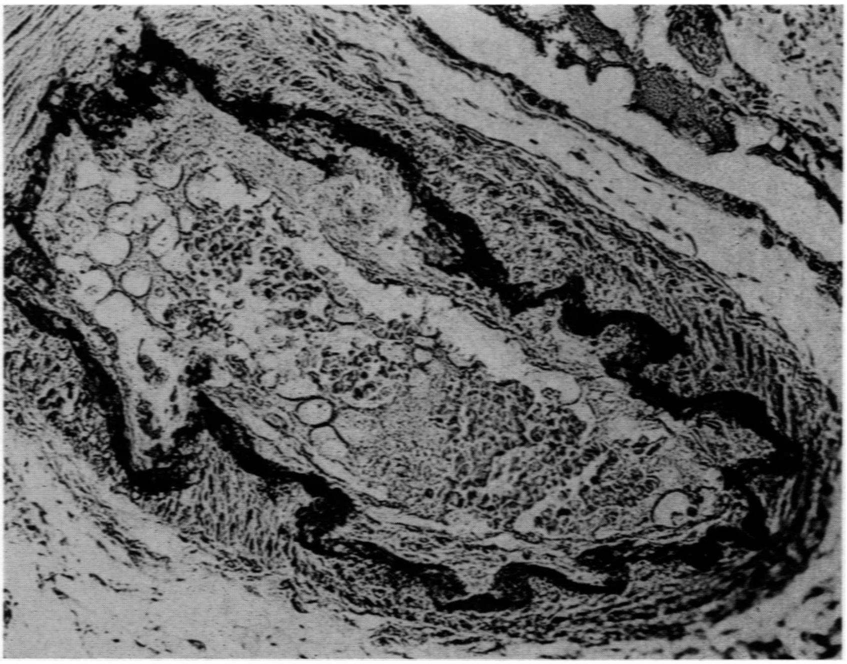

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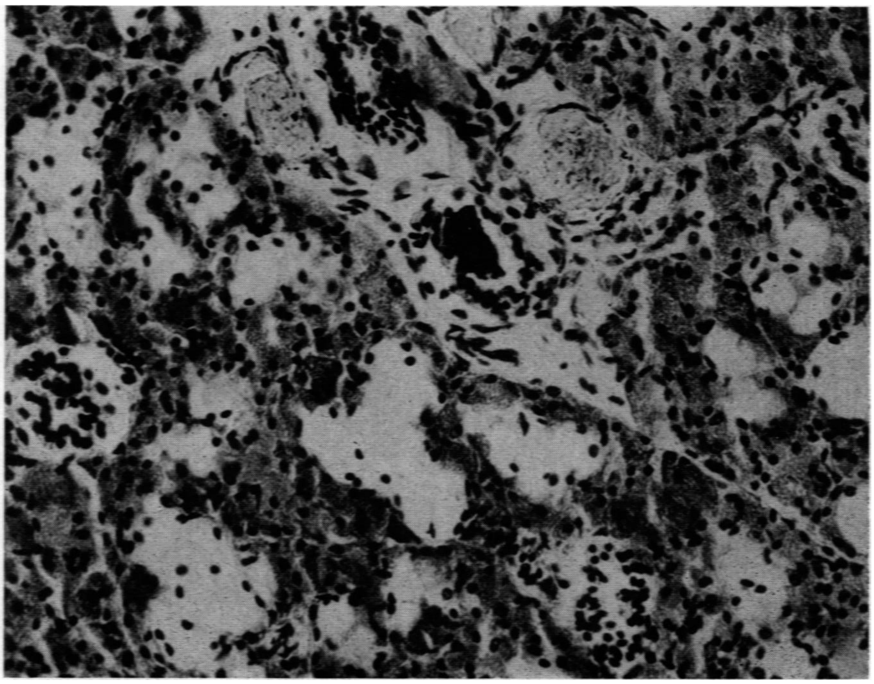
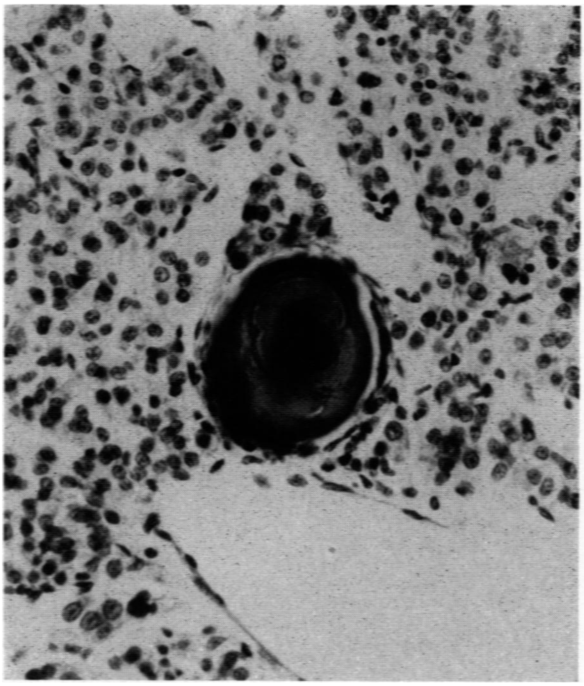


FIG. 9. Small artery from tissue adjacent to an axillary lymph node. The internal elastic lamina is calcified. Von Kossa stain. × 220.

FIG. 10. Submaxillary gland. A mineral deposit is found in a small duct. Hematoxylin and eosin stain. × 150.



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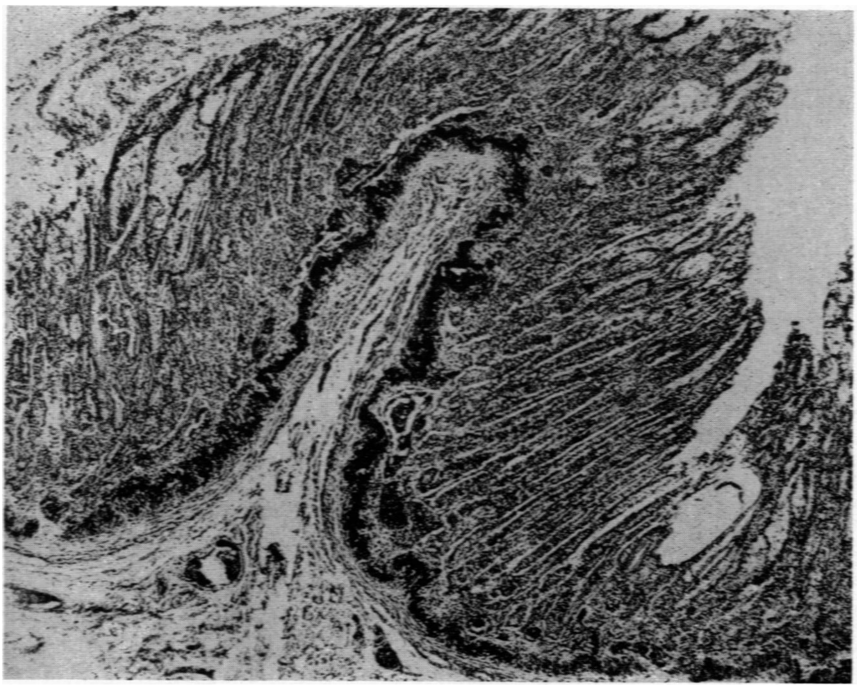

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FIG. 11. Adrenal. A calcified mass in the inner portion of the cortex. Hematoxylin and eosin stain. × 60.

FIG. 12. Stomach. Note the extensive calcification in the mucosa. Von Kossa stain. × 80.